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Electronics of a Flow Cytometer

◀ [Flow Cytometry Fundamentals](#)

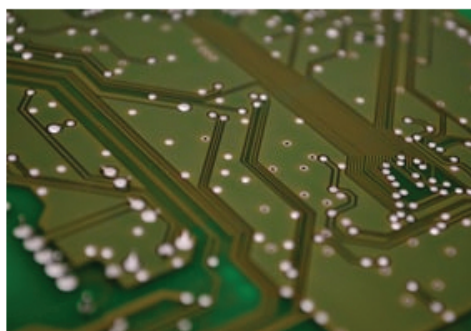
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Electronics of a Flow Cytometer

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The electronics system in a flow cytometer is responsible for digitizing and processing the photocurrent from the detector and saving it for subsequent analysis.

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Follow the photons

The photons that are emitted when the laser hits the cell as it passes through the interrogation point are detected by the photomultiplier (PMD) or photodiode (PD). These photons can come from light being scattered by the cell or by fluorescence emission of fluorophores associated with the cell. Once in the detector, the photons are converted to electrons, and the signal is multiplied proportionately. The signal exits the detector as an electric current (also called **photocurrent**), and this is the point where the signal enters the electronics system. A simplification of the path of the photons as they travel through the electronics system can be seen in **Figure 1**. After exiting the detector the electric current travels to the amplifier (amp) where it is amplified and converted to a voltage pulse. This pulse is then converted to a digital number via the analog-to-digital converter (ADC). The digital number is transferred to the computer and becomes the data that you will analyze. Of course this is a dramatic simplification of the process, and there are some key points described in this section that you will need to understand about the data in order to accurately interpret the results of your experiment.

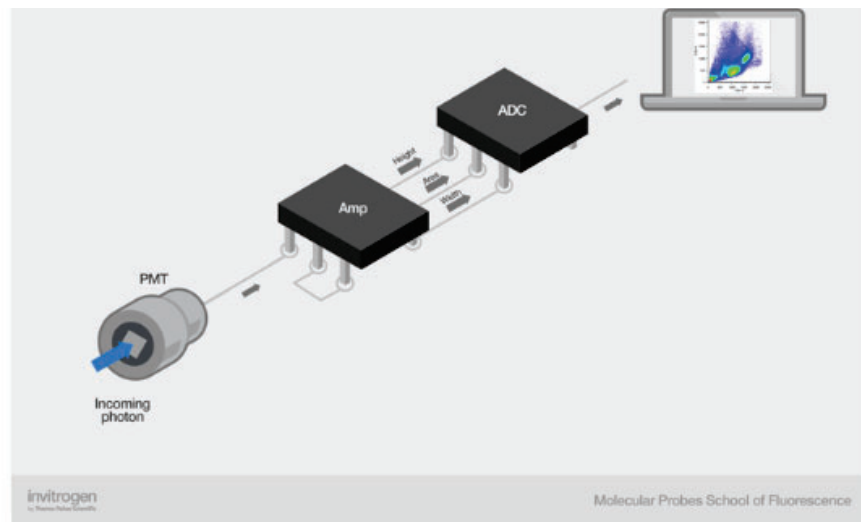


Figure 1. Path of the photons in the electronics system.

Understanding the voltage pulse

The **voltage pulse** (also called a signal pulse) is created when the cell reaches the interrogation point and crosses the path of the laser. The number of photons emitted is proportional to the fluorescent light generated after the cell passes through the laser as demonstrated by the pattern shown in **Figure 2**. These photons are converted to electrons in the detector, which is why these are called voltage pulses. The size of the voltage pulse also depends on the PMT voltage or pre-amplifier gain and the amplification factor (also called the amplification gain). Signals can be amplified by applying a voltage to the PMTs, thus creating a greater electrical current, or by increasing the amplification gain. Amplifier settings can be linear or logarithmic (Lin or Log). Log amplification is often used to separate negative from dim positive signals, whereas Lin amplification is often used to amplify scatter and fluorescent parameters.

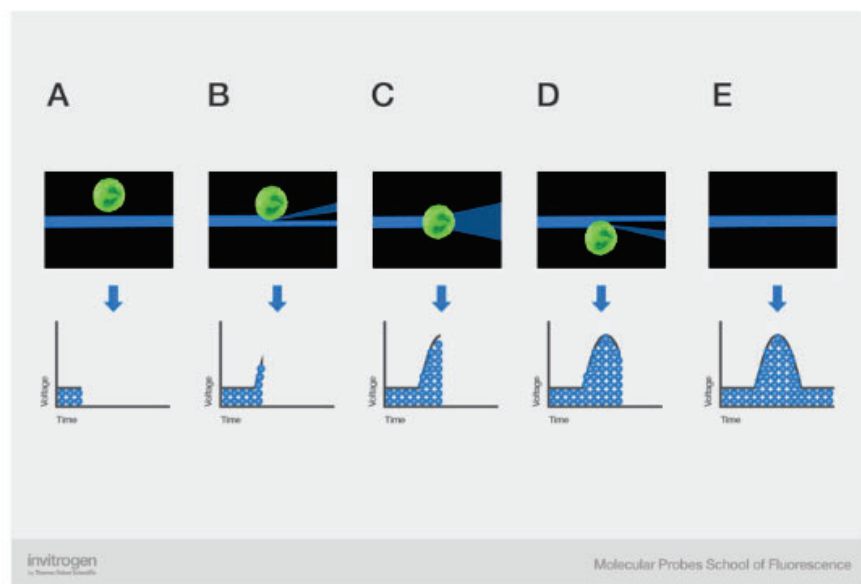


Figure 2. Creation of the voltage pulse as a flowing cell interacts with the laser at the interrogation point. (A) Before the particle enters the interrogation point, baseline signal exists in the system. The curve represents the level of photons being emitted. (B) As the cell begins to cross the path of the laser beam, light is emitted or scattered so that an increase in signal intensity is seen. (C) Full illumination of the cell within the

laser beam results in maximum signal height of the pulse being collected. (D) As the cell begins to exit from the interrogation point the signal decreases. (E) Once the cell is completely past the laser beam, the signal returns to the baseline level.

Anatomy of the voltage pulse

The voltage pulse has three attributes (Figure 3). The pulse **height** is the maximum peak of light collected. The **full pulse width** is the time from the start of the pulse to the end of the pulse. The pulse **area** is the integral of the height over width (time). Each of these three measurements provides different information about the cell and is used to answer a specific experimental question.



Figure 3. Anatomy of the voltage pulse.

Interpreting the voltage pulse of scatter

Forward scatter light (FSC), also called low-angle light scatter, is the amount of light that is scattered in the forward direction as a laser light strikes the cell. The magnitude of forward scatter is roughly proportional to the size of the cell, which means the voltage pulse of the forward scatter will reflect the relative size of the cell. As shown in Figure 4, the amount of light scattered in the forward direction is less intense with small cells than the light scattered by larger cells and the resulting voltage pulse will also be smaller.

Side scatter light (SSC) or light that is scattered at larger angles, is caused by granularity and structural complexity inside the cell or on the cell surface. Increased cell complexity results in more light scatter and larger voltage pulses (not illustrated).

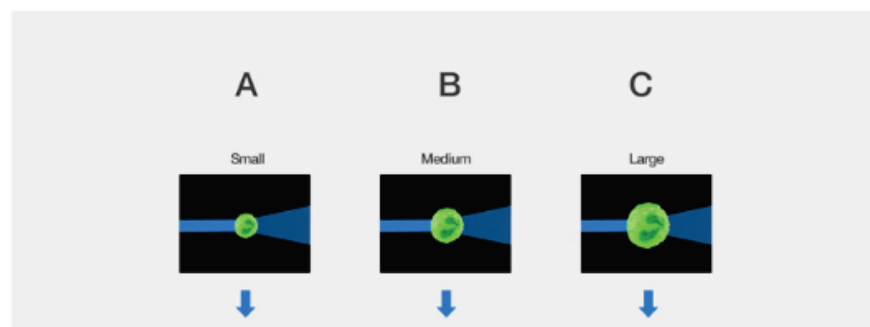




Figure 4. Voltage pulse for forward scatter on three different sizes of cells.

Interpreting the voltage pulse of fluorescence emission

As with FSC and SSC, the fluorescent light emitted by the cell as it crosses the laser beam will result in a voltage pulse. The amount of fluorescence emitted by the fluorophores associated with the cell is dependent on a couple of factors. The first factor is the number of fluorophores associated with the cell. For example, the cell could have low expression of the surface protein that you are detecting so that there are very few fluorescent antibody conjugates bound to the surface. Another example could be that a dead cell dye has bound in high quantities because the cell is no longer alive. Both of these examples would have different levels of fluorescence associated with the cell. Examples of the voltage pulses from three cells with different numbers of the same fluorophore label are illustrated in Figure 5.

The second factor that impacts the level of the fluorescence emission is the brightness of the fluorophores. Not all fluorophores are created equal when it comes to how much fluorescence is emitted. For example, some fluorophores have structures that lend themselves to much brighter fluorescence, like phycoerythrin (PE), which is a large protein from red algae. Other fluorophores are very small organic ring structures like fluorescein, which is significantly less bright than PE.

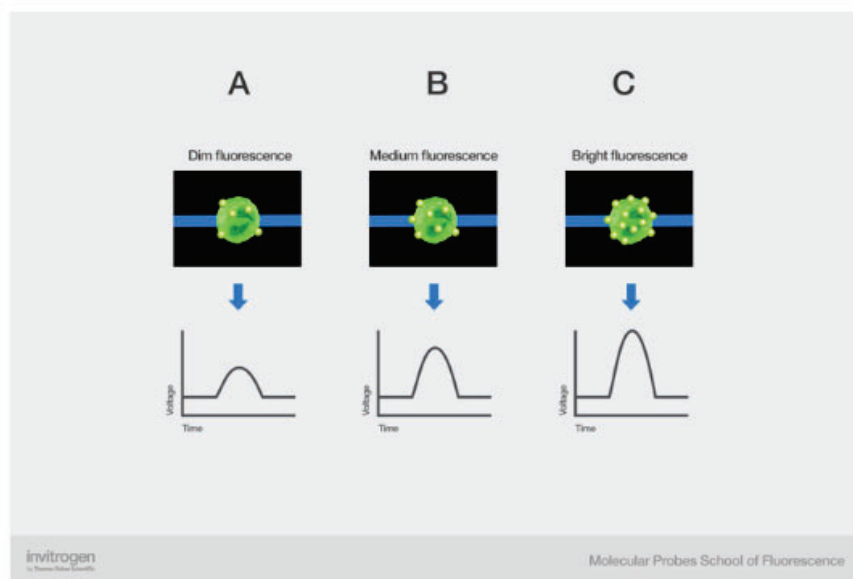


Figure 5. Voltage pulse for fluorescence emission on three different cells with varying numbers of the same fluorophore.

The fate of the voltage pulse (or binning)

As described above, once the voltage pulse has been created it will go through an amplification process and a digitization process. The exact order of these processes depends

on the flow cytometer you are using and this will be discussed in the next two sections. Regardless of the flow cytometer, however, the final fate of the voltage pulse data will be the same. After amplification and digitization, the voltage pulse data will go through a process called **binning**. In this process (**Figure 6**), the voltage pulse data (height, full pulse width, and area) for each cell is assigned to a bin, depending on its value. Every parameter detected for that specific cell (i.e., FSC, SSC, fluorescence of each channel) has a voltage pulse that will be assigned to a bin for that parameter.

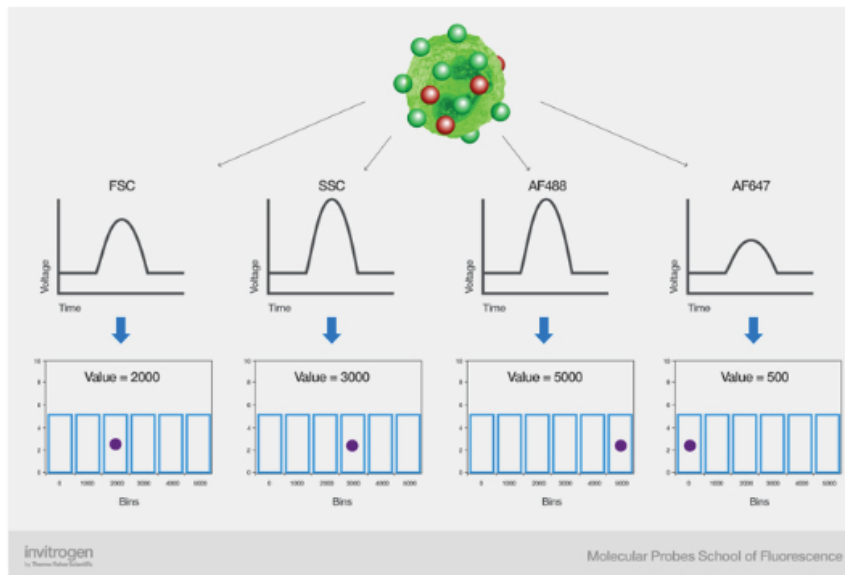


Figure 6. Voltage pulse binning for all parameters associated with a single cell.

As the data for all of the cells are collected and binned during this process, the distribution of the data begins to resemble a population distribution curve or a histogram (**Figure 7**). The number of bins depends on the sampling rate and resolution of the ADC.

Through the electronics of the instrument, the data is digitized (converted from analog to digital), and the final output of a cell sample analysis is a file in the standard flow cytometry format, an FSC digital data file, which is ready for analysis. The path from cell to data generation is now complete, and the exciting (and sometimes excruciating!) task of data analysis can begin.

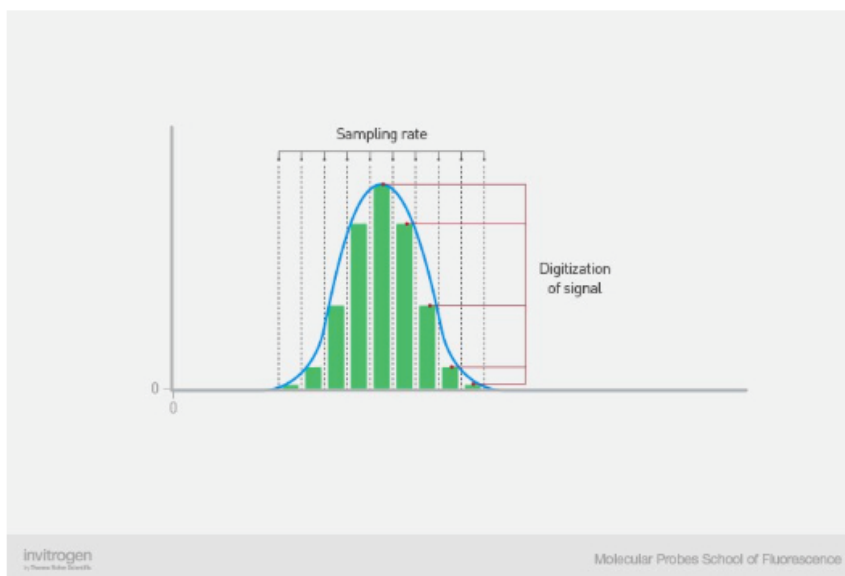


Figure 7. Distribution of the voltage pulse data during the binning process.

Flow cytometers—old versus new

The signal from the PMT is very low, and typically goes through some pre-amp circuitry to amplify the signal before processing. While there are still pulses generated in some earlier-generation flow cytometers that use an analog pulse processing path (Figure 8), modern cytometers use a digital signal processing path (Figure 9). In this pathway, after the amplification the signal pulse is sampled at some rate (between 10 and 25 MHz on commercial systems), and with each sampling the pulse is digitized based on the analog-to-digital converter (ADC) resolution (ranging from 14 to 24 bits).

Analog-to-digital converter (ADC) resolution increases with each new instrument generation

Newer instruments can handle larger data sets and convert at higher bit rates (24 versus 10 bit ADC). For the researcher, this means that higher data resolution can be obtained from the sample when analyzed on an instrument with a 24-bit ADC as opposed to a 10-bit ADC. The ADC resolution is a measure of the number of discrete "bins" or levels that the data can be placed into. For example, an 18-bit ADC has $2^{18} = 262,144$ different bins that can be used to digitize the given value. At a given sampling point (represented by a rectangle in Figure 7, the ADC measures the value and places it in one of these bins. At the next sampling point the process is repeated, to give a measure of the whole pulse over time. More bins mean there is more "resolution" in the data, and flow cytometer manufacturers have taken the opportunity to more realistically distribute the bins along the log scale to better reflect this distribution.

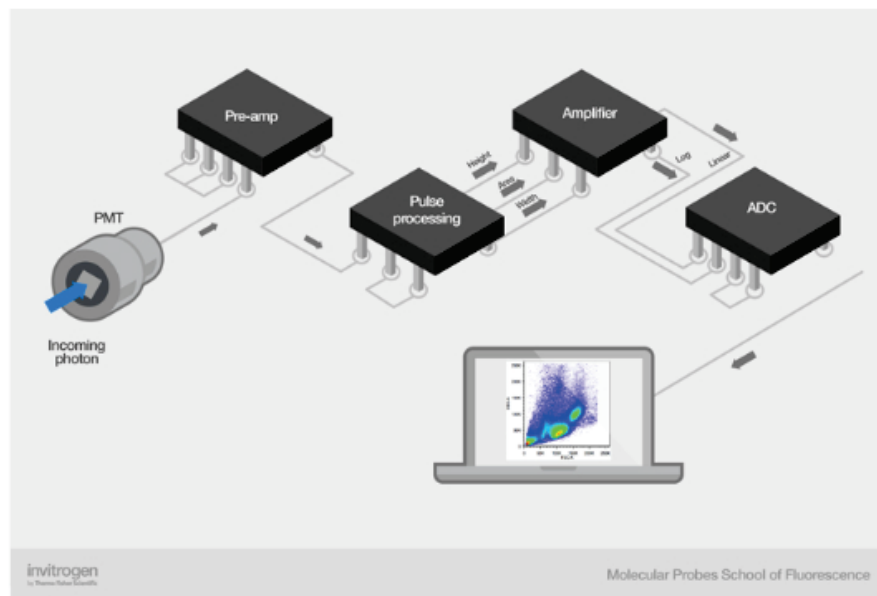
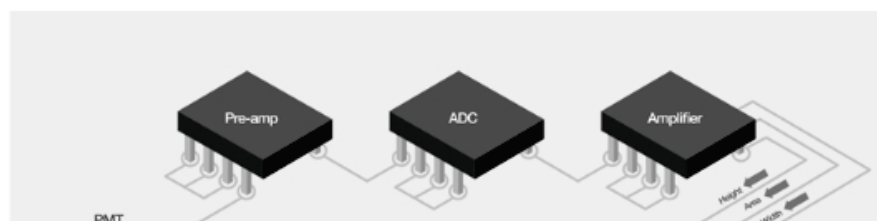


Figure 8. Signal digitization in older flow cytometers.



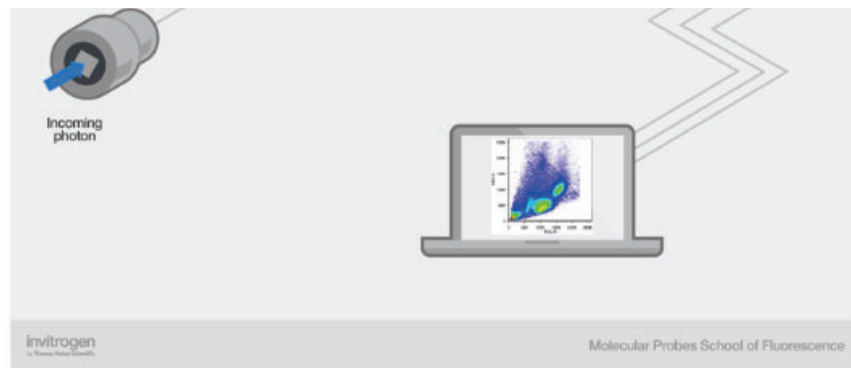


Figure 9. Signal digitization in newer flow cytometers.

Summary

The electronics system acts as the brains of the flow cytometer, converting the photons to electrons, which are then converted from analog to digitized data. All of the data associated with each individual cell are binned and stored in digital data file that can be read and analyzed using the appropriate analysis software.

Resources

[Flow Cytometry application notes](#)
[Flow Cytometry Panel Builder](#)
[Fluorescence SpectraViewer](#)

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Recommended reading

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